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## **Heart rate variability parameters in horses distinguish atrial fibrillation from sinus rhythm before and after successful electrical cardioversion**

Broux, B ; De Clercq, D ; Decloedt, A ; Ven, S ; Vera, L ; van Steenkiste, G ; Mitchell, Katharyn ; Schwarzwald, Colin ; van Loon, G

**Abstract:** **BACKGROUND:** Atrial fibrillation (AF) is the most common pathological arrhythmia in horses. After successful treatment, recurrence is common. Heart rate monitors are easily applicable in horses and some devices offer basic heart rate variability (HRV) calculations. If HRV can be used to distinguish between AF and sinus rhythm (SR), this could become a monitoring tool for horses at risk for recurrence of AF. **OBJECTIVES:** The purpose of this study was to assess whether in horses AF (before cardioversion) and SR (after cardioversion) can be differentiated based upon HRV parameters. **STUDY DESIGN:** Cohort study with internal controls. **METHODS:** Six HRV parameters were determined in 20 horses, both in AF and in SR, at rest (2- and 5-min and 1- and 4-h recordings) and during exercise (walk and trot, 2-min recordings). Time-domain (standard deviation of the NN intervals, root mean squared successive differences in NN intervals and triangular index), frequency domain (low/high frequency ratio) and nonlinear parameters (standard deviation of the Poincaré plot [SD]1 and SD2) were used. Statistical analysis was done using paired Wilcoxon signed rank tests and receiver operating characteristic curves. **RESULTS:** HRV was higher during AF compared to SR. Results for the detection of AF were good (area under the receiver operating characteristic curve [AUC] 0.8-1) for most HRV parameters. Root mean squared successive differences in NN intervals and SD1 yielded the best results (AUC 0.9-1). Sensitivity and specificity were high for all parameters at all recordings, but highest during exercise. Although AUCs improved with longer recordings, short recordings were also good (AUC 0.8-1) for the detection of AF. In horses with frequent second degree atrioventricular block, HRV at rest is increased and recordings at walk or trot are recommended. **MAIN LIMITATIONS:** Animals served as their own controls and there was no long-term follow-up to identify AF recurrence. **CONCLUSIONS:** AF (before cardioversion) and SR (after cardioversion) could be distinguished with HRV. This technique has promise as a monitoring tool in horses at risk for AF development.

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**Heart rate variability parameters in horses distinguish atrial fibrillation from sinus rhythm before and after successful electrical cardioversion.**

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**Keywords:** arrhythmia, cardiology, equine, heart rate monitor

**Summary**

**Background:** Atrial fibrillation (AF) is the most common pathological arrhythmia in horses. After successful treatment, recurrence is common. Heart rate monitors are easily applicable in horses and

some devices offer basic heart rate variability (HRV) calculations. If HRV can be used to distinguish

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between AF and sinus rhythm (SR), this could become a monitoring tool for horses at risk for recurrence of AF.

**Objectives:** The purpose of this study was to assess whether in horses AF (before cardioversion) and SR (after cardioversion) can be differentiated based upon HRV parameters.

**Study design:** Cohort study with internal controls.

**Methods:** Six HRV parameters were determined in 20 horses, both in AF and in SR, at rest (2- and 5-minute and 1- and 4-hour recordings) and during exercise (walk and trot, 2-minute recordings). Time-domain (SDRR, RMSSD and Triangular index), frequency domain (LF/HF ratio) and nonlinear parameters (SD1 and SD2) were used. Statistical analysis was done using paired Wilcoxon Signed Rank Tests and Receiver Operating Curves.

**Results:** HRV was higher during AF compared to SR. Results for the detection of AF were good (AUC 0.8-1) for most HRV parameters. RMSSD and SD1 yielded the best results (AUC 0.9-1). Sensitivity and specificity were high for all parameters at all recordings, but highest during exercise. Although AUCs improved with longer recordings, short recordings were also good (AUC 0.8-1) for the detection of AF. In horses with frequent second degree atrioventricular block, HRV at rest is increased and recordings at walk or trot are recommended.

**Main limitations:** Animals served as their own controls and there was no long term follow up to identify AF recurrence.

**Conclusions:** AF (before cardioversion) and SR (after cardioversion) could be distinguished with HRV. This technique has promise as a monitoring tool in horses at risk for AF development.

## Introduction

Atrial fibrillation (AF) is the most common clinically important arrhythmia in horses. Reported prevalence ranges between 0.3 and 2.5% of the population, with an overrepresentation in Standardbreds and Warmblood horses [1-3]. Timely diagnosis and treatment are important and definite diagnosis is made with electrocardiography (ECG) [4]. Successful restoration of sinus rhythm (SR) by electrical or pharmacological cardioversion can often be attained, but reported recurrence

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rates range between 16 and 43% [5-9]. Diagnosis of early recurrence cannot always be performed by lay people and may require frequent veterinary visits.

Heart rate variability (HRV) describes and quantifies the beat-to-beat variability and the long-term variation in heart rate [10]. Under the influence of both the autonomic nervous system and the neuroendocrine system, cyclical and beat-to-beat variations in heart rate are normal in the healthy individual. Decrease in parasympathetic tone and increase in sympathetic tone result in a decrease in short term HRV. In human medicine, HRV is often used to predict prognosis in various cardiac disease states [11]. In horses it has been used to study the autonomic regulation of the heart and it is frequently used in animal welfare studies, quantifying stress and pain [12-17]. Since arrhythmia, especially AF, leads to an increased variation in beat-to-beat intervals, HRV parameters describing short-term (beat-to-beat) variability are expected to be increased compared to horses in SR. An increase in HRV in human patients with AF has been described and HRV parameters have been used to diagnose arrhythmias [18-22]. If HRV parameters can also be used to differentiate AF from SR in horses, a heart rate monitor or smartphone app with automated HRV calculations would be an accessible diagnostic tool for both veterinarians and horse owners to monitor their horses for the presence of AF. The purpose of the current study was to determine whether HRV parameters at rest, during exercise at different paces and at different recording durations, differ between horses in AF and in SR, before and after successful transvenous electrical cardioversion (TVEC), respectively.

## Materials and Methods

### *Experimental design and population*

In this cohort study, each horse was used as its own internal control. 20 Warmblood horses presented to the Department of Equine Internal Medicine, Faculty of Veterinary Sciences, Ghent University, for the treatment of AF using transvenous electrical cardioversion (TVEC) were used [23]. Inclusion criteria were electrocardiographic confirmation of AF and successful electrical cardioversion. Horses ranged between 5 and 15 years, with a mean $\pm$ SD age of 10 $\pm$ 3 years. Two stallions, 11 geldings and 7 mares were included. Echocardiography was performed in all horses in AF. A resting ECG and a standardized exercise ECG were taken from each horse before (AF) and 5 days after (SR) TVEC.

### *Electrocardiography*

In all 20 horses an ECG was recorded using a Televet100 recording system<sup>a</sup>. Four self-adhesive electrodes were placed underneath a girth<sup>b</sup>. The right arm electrode was positioned 15 cm right of the withers, the left leg electrode caudal to the left elbow on the thorax and the left arm electrode 10 cm above the left leg electrode resulting in a modified base-apex configuration [24; 25]. A reference electrode was placed on the left side of the withers. All electrodes were connected to the recording device, placed in the girth.

For resting ECG horses were confined in a stable with access to feed and water. All horses had been stabled in this environment for at least 1 day in order to minimize environmental stress factors. The standardized lunging exercise test consisted of 5 minutes of walk, followed by 10 minutes of trot, 2 minutes of walk, 4 minutes of canter, 1 minute of gallop and 10 minutes of walking. The ECG recordings were analyzed offline using dedicated software<sup>c</sup>. Standard gain (10 mm/mV) and paper speed (25 mm/s) were used. RR intervals were automatically calculated by the software program (RR detection Equine), peak detection was set on negative (S wave). Three different leads were displayed simultaneously to improve ECG interpretation, but S peak detection was always performed in lead 2 (between the right arm (-) and left leg (+) electrode). All ECGs were visually inspected and corrected manually if necessary. Interference, electrical interruptions or software errors in peak detection were corrected or otherwise removed from the database. All cardiac arrhythmias were documented.

### *Heart rate variability*

RR intervals were exported into a text file and subsequently imported into commercial software for HRV analysis<sup>d</sup>. Each text file was blinded by giving a random number before analysis. For the resting ECG, 15 minutes after starting the ECG recording, a 2-minute, 5-minute, 1-hour and 4-hour recording were analyzed. For the exercise ECGs, a 2-minute recording at walk and trot, starting 2 minutes after the onset of the walking and trotting phase, were analyzed. Six HRV parameters were used for further analysis. Three time domain parameters: SDNN (standard deviation of the RR intervals), RMSSD (root mean squared successive differences in RR intervals) and Triangular Index (TI) (integral of RR interval histogram divided by height of the histogram); 1 frequency domain parameter: LF/HF ratio

(the ratio of the low frequency band over the high frequency band); and 2 nonlinear parameters: SD1 and SD2 (standard deviation of the Poincaré plot perpendicular to and along the line of identity, respectively) (Fig.1) [11]. For frequency domain analyses, frequency values for horses were set at 0.001-0.005Hz for the very low frequency band (VLF), 0.005-0.07Hz for the low frequency band (LF) and 0.07-0.5Hz for the high frequency band (HF) [10-12]. There were no filters nor artifact corrections applied.

### *Data analysis*

Statistical analysis was performed using dedicated software<sup>c</sup>. Descriptive statistics, quantile-quantile plots and Shapiro-Wilk tests were used to check for normality of the data. Data were not normally distributed. Paired Wilcoxon Signed Rank Tests were used to detect significant differences between observations. Significance was set at  $P < 0.05$ . Receiver operating characteristic (ROC) curves were used to study the performance of a parameter as a discriminatory variable for the detection of AF. The coordinate points of the ROC curves were used to determine the most fitting cut off values for each parameter at each recording. Cut off values were chosen to maximize sensitivity whilst retaining good ( $\geq 80\%$ ) specificity, where possible.

### **Results**

Echocardiography revealed mild to moderate mitral (11/15; 3/15), aortic (5/15, 0/15), tricuspid (7/15; 3/15) and pulmonary (1/15; 0/15) valve regurgitation in 15 horses and mild dilatation of the right (3/3) and left (2/3) ventricle and the right (1/3) and left (1/3) atrium in 3 of those horses. In 5 horses, no structural or functional echocardiographic abnormalities were detected.

All HRV values were significantly different in SR compared with AF (Table 1). In 11 horses in SR at rest, occasional arrhythmias ( $<1\%$  of RR intervals) such as supraventricular premature depolarizations (SVPD) (8/11), second degree atrioventricular block (2AVB) (6/11), ventricular premature depolarizations (VPD) (2/11) and ventricular tachycardia (VT) (1/11) increased HRV, but not above the cut off values for AF. In 6 horses with frequent episodes of sinus arrhythmia, HRV parameters were within the limits of the other horses in SR. Where more arrhythmias (1-10% of RR intervals,

n=2) were present, HRV values increased and sometimes hampered differentiation between SR and AF at rest. In 2 horses with very frequent (12% and 18% of the RR intervals) 2AVB, HRV values at rest in SR approached or even exceeded those in AF. When these horses were exercised, 2AVB disappeared and HRV values decreased to within the range of the other horses in SR (Fig.1).

Area under the ROC curves and cut off values with their sensitivity and specificity for each parameter at each recording duration at rest or during exercise, are summarized in Table 2. AUCs were high (>0.8) for all parameters at rest and very high (>0.9) during exercise. RMSSD and SD1 overall had the highest sensitivity (100%) and specificity (80-100%). From a 2-minute walk recording, RMSSD and SD1 had an AUC of 1 and 100% sensitivity and specificity.

## Discussion

The current study demonstrated that AF (before TVEC) and SR (after TVEC) can be differentiated based upon HRV parameters. Cut off values with good sensitivity and specificity were determined for SDNN, RMSSD, TI, LH/HF ratio, SD1 and SD2 at rest and during walking and trotting. At rest, good results with high sensitivity and specificity were obtained, even for short recordings. Light exercise (walk and trot), however, led to the most reliable cut off values.

In this study, HRV is not used in its traditional way to study the autonomic control of the heart, but to quantify the variation in RR intervals to detect the presence of a certain arrhythmia. Ranges of HRV parameters are therefore different and not comparable with values published in previous studies using HRV as a tool to study autonomic tone. Higher variability represents more arrhythmia. In horses, due to their high parasympathetic tone, arrhythmias are common. AF, however, leads to an extremely irregular heart rhythm. The variability in heart rate was therefore expected to be higher when horses were in AF compared to SR. In human medicine, several algorithms using HRV parameters are implemented in automatic devices detecting AF [18-22].

Apart from AF, other arrhythmias might also increase HRV. We chose to study horses before and after TVEC as arrhythmias, such as SVPD, were expected to occur more frequently in the post



cardioversion period, making the differentiation between AF and SR challenging. The presence of occasional (<1% of the RR intervals) arrhythmias did not increase HRV above the cut-off values for AF but very prevalent arrhythmias might make the differentiation between SR and AF more difficult. In this study, frequent 2AVB resulted in increased HRV values but 2AVB was abolished at walk or trot. Most HRV parameters are based on beat-to-beat variability in RR interval. In 2AVB, a blocked beat leads to a major increase in RR interval, while other arrhythmias, such as premature beats or sinus arrhythmia, lead to smaller changes, with less influence on HRV. In horses with frequent 2AVB or other arrhythmias, care should thus be taken when drawing conclusions from recordings at rest and suspicious recordings should be repeated while walking or trotting the horse. If HRV remains increased during exercise, a pathological arrhythmia is likely to be present. However, more studies are necessary to assess the influence of different pathological arrhythmias on HRV.

Based on the results of a preliminary study investigating 144 different HRV parameters, data available from literature and availability of parameters on different heart rate monitors, HRV devices and internet applications, we used 6 HRV parameters for our study [11; 26]. The simplest HRV value to calculate is SDNN. This is a time-domain parameter expressing overall HRV [11]. It is easy to use and readily available on various commercial heart rate monitors. Good results were obtained for SDNN, especially during exercise. At rest, the presence of frequent 2AVB can lead to false positives. RMSSD is mainly used to represent short term HRV (vagal influences) [10; 13]. Cut off values for this parameter had a sensitivity of 100% and a specificity 80-100%, identifying all cases of AF with only a small chance of false positives. RMSSD is routinely used and available, which makes it an excellent tool for home monitoring. TI was chosen to include a geometrical time domain parameter and represents overall HRV. It has the advantage of being less sensitive to the analytical quality of the RR interval, excludes outliers and covers many problems of artifacts [10]. Its major disadvantage is the need for a relatively large number of RR intervals to construct the histogram. In horses, recordings of at least 60 minutes are advised to ensure the correct performance of geometric methods for studying autonomic tone [10]. SD1 and SD2 are derived from nonlinear dynamics and based on the Poincaré Plot of the RR intervals. While excellent results were obtained for SD1, SD2 yielded moderate results.

Since SD2 mainly represents long-term HRV, and beat-to-beat variability is averaged out, it was expected to be less influenced by AF. The Poincaré plot display the RR intervals against their previous RR interval. Arrhythmias will thus cause a dispersion of the plot towards the upper left and lower right area of the graph, increasing SD1 in particular (Fig.1). Finally, the ratio between LF and HF components was used as a power spectral density analysis method of HRV. In contrast to the other parameters, a value lower (and not higher) than the cut off value indicates the presence of AF. For short recordings at rest moderate sensitivity and specificity was obtained, so longer recordings at rest or recordings during exercise are necessary.

Cut off values determined in this study were chosen to optimize sensitivity whilst retaining good specificity. As AF may be associated with potentially dangerous ventricular rhythms during exercise and because early diagnosis positively affects treatment outcome, our focus was to identify all horses with AF, even if this would include some false positives [4]. Furthermore, if RR identification is correct and no artifacts or electrical interference are present on the ECG, false positives will most likely arise from horses with frequent arrhythmias. If these horses remain positive during exercise (walk and trot), it is likely that a pathological arrhythmia is present and further examination (ECG) is indicated.

This study demonstrated that AF can be distinguished from SR before and after TVEC using HRV. Because arrhythmias, especially SVPD, often appear more frequently in the post-cardioversion period, this differentiation was probably more challenging in the current study than if a control group of normal horses had been used. Therefore, it seems highly likely that HRV monitoring can be used to monitor horses at risk for developing AF, such as horses with mitral valve regurgitation, a dilated atrium, SVPD or horses after successful AF treatment. However, an important limitation of this study is that we did not include long term follow-up until recurrence.

Heart rate monitors can automatically generate some basic HRV calculations, mostly SDNN, RMSSD and/or SD1. The majority of equine HRV studies have used Holter recordings, but equine heart rate

monitors have also been used [14; 27; 28]. This is an accessible tool for both veterinarians and horse owners. In the current study, we used very short recording durations (2 and 5 minutes). Our study demonstrated only slight improvements in sensitivity and specificity when using longer (1 or 4 hour) recordings. Heart rate monitors have the benefit of being affordable and easy to use, but they also have inherent limitations. Heart rate monitors automatically detect the R-peak of the ECG. In horses, the T wave can be very pronounced and systems based on detecting R peaks by looking for sharp deflections from the baseline, can erroneously register T waves. Movement and electrical artifacts are common, especially during exercise, and may lead to erroneous R peak registrations. Most heart rate monitors are not validated for the use in horses and significant differences between results from heart rate monitors and ECG recordings have been shown [28]. Further studies are necessary to assess whether heart rate monitors can correctly detect RR intervals in horses and whether they can differentiate between horses in AF and in SR.

We did not include exercise at high speed in this study. The exercise tests in SR were performed 5 days after TVEC and only low-level exercise was performed. In addition, based upon HRV parameters, discriminating between AF and SR at high heart rates is expected to be more difficult. In human medicine, HRV after cardioversion is used to predict AF recurrence. This was beyond the scope of the current study, but warrants further attention.

## **Conclusions**

This study demonstrated that HRV is significantly increased when horses are in AF, compared to when they are in SR. HRV could potentially be used as a tool for early detection of AF in horses judged to be at risk. ECG would then be required to confirm the diagnosis.

## **Authors' declaration of interests**

No competing interests have been declared.

## **Ethical animal research**

The authors confirm that approval from their institute's ethics committee is not required for this non-invasive clinical study. Owners gave consent for their animals' inclusion in the study.

## **Source of funding**

None.

## **Authorship**

B. Broux contributed to study design, study execution, data analysis and interpretation, and preparation of the manuscript. D. Declercq contributed to study execution and preparation of the manuscript. A. Decloedt contributed to study execution, data analysis and interpretation, and preparation of the manuscript. S. Ven, L. Vera and G. van Steenkiste contributed to study execution. K. Mitchell and C. Schwarzwald contributed to data analysis and interpretation, and preparation of the manuscript. G. van Loon contributed to study design, data analysis and interpretation, and preparation of the manuscript. All authors gave their final approval of the manuscript.

## **Manufacturers' addresses**

a: Televet100, Engel Engineering Services GmbH, Heusenstamm, Germany.

b: Mainat Vet, Barcelona, Spain.

c: Televet100 software version 6.0, Engel Engineering Services GmbH, Heusenstamm, Germany.

d: Kubios 2.2, University of Eastern Finland, Finland.

e: IBM SPSS Statistics 24, IBM corp., United States.

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### Figure Legends:

**Fig 1:** RMSSD of 20 Warmblood horses in atrial fibrillation (AF; dotted lines) and in sinus rhythm (SR; solid lines) during a 2 minute ECG recording at rest and walk. Cut off RMSSD values for the detection of atrial fibrillation (302ms at rest and 92ms at walk) are represented by the horizontal lines. Notice that 2 horses with frequent second degree atrioventricular block in SR (black lines) show high RMSSD at rest, above the cut off for AF. At walk, both horses show an RMSSD below the cut off value.

**Fig 2A and 2B:** Poincaré plots of a horse at rest during atrial fibrillation (A) and sinus rhythm (B). RR intervals are plotted against their preceding RR interval. SD1 and SD2 are calculated as the standard deviation of the Poincaré plot perpendicular to and along the line of identity, respectively. Notice the dispersion of the values towards the upper left and lower right corner, increasing SD1 in case of atrial fibrillation. In case of sinus rhythm, notice how 7 second degree atrioventricular blocks, increasing SD1, are visible in the upper left and lower right corner of the graph.

**Table 1:** Median and range of 6 heart rate variability parameters in 20 warmblood horses in sinus rhythm and in atrial fibrillation at rest and during exercise.

Phase	Variable	SR			AF			P- value
		Median	Min	Max	Median	Min	Max	
2 min rest	SDNN (ms)	158	22	901	462	240	1111	0.001
2 min rest	RMSSD (ms)	89	18	1472	689	325	1745	0.001
2 min rest	TI	14	4	21	22	13	34	<0.001
2 min rest	SD1 (ms)	63	13	1051	490	21	1243	0.001
2 min rest	SD2 (ms)	201	22	741	440	247	475	0.002
2 min rest	LF/HF ratio	0.93	0.10	24.08	0.19	0.06	1.45	0.001
5 min rest	SDNN (ms)	154	65	781	469	306	1057	0.001
5 min rest	RMSSD (ms)	102	29	1215	675	375	1686	0.001
5 min rest	TI	17	8	40	34	24	45	<0.001
5 min rest	SD1 (ms)	75	21	862	478	266	1196	0.001
5 min rest	SD2 (ms)	196	63	838	478	308	902	0.002
5 min rest	LF/HF ratio	2.40	0.09	70.23	0.16	0.85	0.43	<0.001
1 hour rest	SDNN (ms)	190	127	768	55	307	1077	<0.001
1 hour rest	RMSSD (ms)	128	47	1057	813	292	1647	<0.001
1 hour rest	TI	29	18	72	58	29	73	<0.001
1 hour rest	SD1 (ms)	91	33	748	575	277	1165	<0.001
1 hour rest	SD2 (ms)	242	156	788	544	327	982	0.001
1 hour rest	LF/HF ratio	2.79	0.17	39.86	0.26	0.13	0.36	<0.001
4 hours rest	SDNN (ms)	231	130	765	627	279	1148	<0.001
4 hours rest	RMSSD (ms)	153	42	1103	886	366	1804	<0.001
4 hours rest	TI	44	23	80	70	46	88	0.001
4 hours rest	SD1 (ms)	109	30	78	627	259	1276	<0.001
4 hours rest	SD2 (ms)	298	181	749	626	298	1003	<0.001
4 hours rest	LF/HF ratio	2.41	0.20	25.85	0.25	0.19	0.37	<0.001
2 min walk	SDNN (ms)	79	24	123	186	93	935	<0.001
2 min walk	RMSSD (ms)	28	7	77	216	106	1195	<0.001
2 min walk	TI	12	4	19	20	16	29	<0.001
2 min walk	SD1 (ms)	20	5	55	153	75	850	<0.001
2 min walk	SD2 (ms)	110	33	172	199	100	1017	<0.001
2 min walk	LF/HF ratio	7.54	0.39	45.82	0.40	0.08	3.62	<0.001
2 min trot	SDNN (ms)	19	7	39	70	39	112	<0.001
2 min trot	RMSSD (ms)	7	3	53	84	48	130	<0.001
2 min trot	TI	4	2	9	13	8	21	<0.001
2 min trot	SD1 (ms)	5	2	37	59	34	92	<0.001
2 min trot	SD2 (ms)	24	8	51	73	43	138	<0.001
2 min trot	LF/HF ratio	5.35	0.22	24.50	0.26	0.08	0.92	<0.001

SR: sinus rhythm, AF= atrial fibrillation, SDNN= standard deviation of the NN intervals, RMSSD = root mean squared successive differences, TI = Triangular Index, SD= standard deviation of the Poincaré plot, LF= low frequencies, HF= high frequencies, ms = milliseconds



**Table 2:** Area under the Receiver Operating Curves (ROC) with cut off values, sensitivity and specificity for 6 heart rate variability parameters in 5 different recording phases for the detection of atrial fibrillation (AF) in 20 horses. A value above the cut off indicates AF, except for \* where it indicates SR.

Phase	Variable	AUC	95% confidence interval		Cut off	Sens	95% confidence interval		Spec	95% confidence interval	
			Lower B	Upper B			Lower B	Upper B		Lower B	Upper B
2 min rest	SDNN (ms)	0.89	0.78	1	248	1	0.8	1	0.8	0.56	0.93
2 min rest	RMSSD (ms)	0.9	0.79	1	302	1	0.8	1	0.8	0.56	0.93
2 min rest	Triangular I	0.92	0.84	1	17	0.9	0.67	0.98	0.8	0.56	0.93
2 min rest	SD1 (ms)	0.9	0.79	1	215	1	0.8	1	0.8	0.56	0.93
2 min rest	SD2 (ms)	0.87	0.75	0.99	283	0.9	0.67	0.98	0.8	0.56	0.93
2 min rest	LF/HF ratio*	0.83	0.7	0.96	0.41	0.95	0.73	1	0.7	0.45	0.87
5 min rest	SDNN (ms)	0.89	0.78	1	279	1	0.8	1	0.8	0.56	0.93
5 min rest	RMSSD (ms)	0.93	0.83	1	367	1	0.8	1	0.9	0.67	0.98
5 min rest	Triangular I	0.9	0.8	1	24	1	0.8	1	0.8	0.56	0.93
5 min rest	SD1 (ms)	0.93	0.83	1	260	1	0.8	1	0.9	0.67	0.98
5 min rest	SD2 (ms)	0.87	0.74	1	299	1	0.8	1	0.8	0.56	0.93
5 min rest	LF/HF ratio*	0.88	0.76	1	0.38	0.95	0.73	1	0.85	0.61	0.96
1 hour rest	SDNN (ms)	0.92	0.82	1	305	1	0.8	1	0.8	0.56	0.93
1 hour rest	RMSSD (ms)	0.94	0.85	1	310	1	0.8	1	0.9	0.67	0.98
1 hour rest	Triangular I	0.88	0.76	0.99	44	0.9	0.67	0.98	0.8	0.56	0.93
1 hour rest	SD1 (ms)	0.94	0.85	1	219	1	0.8	1	0.9	0.67	0.98
1 hour rest	SD2 (ms)	0.88	0.76	0.99	430	0.85	0.61	0.96	0.85	0.61	0.96
1 hour rest	LF/HF ratio*	0.96	0.87	1	0.39	1	0.8	1	0.95	0.73	1
4 hour rest	SDNN (ms)	0.93	0.83	1	350	0.94	0.73	1	0.85	0.61	0.96
4 hour rest	RMSSD (ms)	0.94	0.85	1	318	1	0.8	1	0.9	0.67	0.98
4 hour rest	Triangular I	0.89	0.78	1	50	0.94	0.73	1	0.75	0.51	0.9
4 hour rest	SD1 (ms)	0.94	0.85	1	225	1	0.8	1	0.9	0.67	0.98
4 hour rest	SD2 (ms)	0.91	0.8	1	394	0.94	0.73	1	0.8	0.56	0.93
4 hour rest	LF/HF ratio*	0.96	0.88	1	0.59	1	0.8	1	0.9	0.67	0.98
2 min walk	SDNN (ms)	0.97	0.93	1	112	0.9	0.67	0.98	0.9	0.67	0.98
2 min walk	RMSSD (ms)	1	1	1	92	1	0.8	1	1	0.8	1
2 min walk	Triangular I	0.96	0.91	1	16	0.9	0.67	0.98	0.9	0.67	0.98
2 min walk	SD1 (ms)	1	1	1	65	1	0.8	1	1	0.8	1
2 min walk	SD2 (ms)	0.91	0.82	1	148	0.85	0.61	0.96	0.8	0.56	0.93
2 min walk	LF/HF ratio*	0.96	0.9	1	1.13	0.95	0.73	1	0.9	0.67	0.98
2 min trot	SDNN (ms)	1	1	1	39	1	0.8	1	1	0.8	1
2 min trot	RMSSD (ms)	1	0.98	1	46	1	0.8	1	0.95	0.73	1
2 min trot	Triangular I	0.99	0.98	1	7.8	1	0.8	1	0.9	0.67	0.98
2 min trot	SD1 (ms)	1	0.98	1	33	1	0.8	1	0.95	0.73	1
2 min trot	SD2 (ms)	0.99	0.96	1	42	1	0.8	1	0.9	0.67	0.98
2 min trot	LF/HF ratio*	0.96	0.9	1	0.94	1	0.8	1	0.9	0.67	0.98

AUC = Area under the curve, Lower B = lower bound, Upper B = upper bound, SDNN= standard deviation of the NN intervals, RMSSD = root mean squared successive differences in NN intervals, TI = Triangular Index, SD= standard deviation of the Poincaré plot, LF= low frequencies, HF= high frequencies, ms = milliseconds



